JOURNAL OF THE
WORLD AQUACULTURE SOCIETY

Vol. 21, No. 1
March, 1990

Baker's Yeast as a Potential Substitute for Live Algae in Aquaculture Diets: Artemia as a Case Study

PETER COUTTEAU, PATRICK LAVENS AND PATRICK SORGELOOS

Laboratory of Aquaculture and Artemia Reference Center, Faculty of Agricultural Sciences, State University of Ghent, Rozier 44, B-9000 Ghent, Belgium

Abstract

In this study baker's yeast was evaluated as a substitute for live *Dunaliella tertiolecta* algae in the culture of the brine shrimp *Artemia*. Consumption of fresh baker's yeast resulted in poor growth and survival of brine shrimp. However, the nutritional value of the yeast significantly improved after complete removal of the yeast cell wall by enzymatic treatment. Baker's yeast was also made digestible for *Artemia* by simple chemical treatment which did not reduce rigidity of the yeast cell. The external mannoprotein layer of the yeast cell wall is probably the major barrier to digestion by *Artemia*. Chemically treated baker's yeast offers promising possibilities as a substitute for algal feeds in aquaculture.

Hatchery production of commercially important organisms such as penaeid shrimp and bivalves requires large volumes of micro-algae, which are used as an essential food for the larval stages. Algae are also used in diets of cultured brine shrimp and rotifers that are in turn used to feed shrimp and fish larvae. Because intensive culture and harvesting of unicellular algae is expensive and labor intensive, replacement of algae with cheap alternative foods would significantly reduce operating costs of aquaculture hatcheries.

Because of their small particle size, high protein content and relatively low production costs, yeasts have been considered as an algal substitute for several species of filter-feeders, e.g., rotifers (Hirayama and Watanabe 1973; Fukusho 1980; Lemilinaire 1984), *Artemia* (Shimaya et al. 1967; Talloen 1978; Johnson 1980; Nimmannit and Assawamunkong 1985) and bivalve molluscs (Epifanio 1979; Urban and Langdon 1984). However, yeasts have not proven to be of consistently high nutritional value. Problems that arise when feeding a yeast monodiet have often been assigned to nutritional deficiencies of the yeast diet (Urban and Langdon 1984; Douillet 1987; Hirayama 1987). However, nutritional value of any diet depends first on its degree of digestibility and second on its content of essential elements. Because yeast cells are known to have a complex and thick cell envelope, poor digestibility may be an important constraint in the use of this Single-Cell Protein (SCP) as a food source in aquaculture in particular and in animal husbandry in general. Two decades ago it was proven that efficient utilization of the protein content of torula yeast (Mitsuda et al. 1969) and *Saccharomyces cerevisiae* (Omstedt et al. 1975) by rats was prevented by the rigid yeast cell wall. More recently, Johnson et al. (1980) reported that the cell wall of *Phaffia rhodozyma* was a barrier to the uptake of the yeast's astaxanthin by rainbow trout.

Several methods have been developed to improve the digestibility of SCP products; mechanical disruption, autolysis, and enzymatic treatment (Kihlberg 1972; Hedenskog and Mogren 1973). However, as a result of these drastic treatments soluble cytoplasmatic contents in the yeast cells are exposed to the environment. As a consequence, yeast nutrients are lost to filter-feeders and, moreover, culture conditions deteriorate due to reduced water quality. The present study documents the low digestibility of fresh baker's yeast fed to *Artemia* and proposes a chemical treatment which

makes the yeast digestible while maintaining cell integrity.

Materials and Methods

Yeast Culture and Preparation

Laboratory cultures of *Saccharomyces cerevisiae* (industrial strain obtained from the company "Gist- en Spiritusfabrieken Bruggeman, N.V.", Belgium) were grown at 30 C, with shaking, in a liquid YEPG medium containing yeast extract (Oxoid, 1% weight/volume), peptone (Oxoid, 1% w/v) and glucose (2% w/v). Yeast was harvested either in the exponential or stationary phase by centrifugation after 12 hours or three days of culture, respectively. Fresh caked baker's yeast was obtained from the same company.

Yeasts were converted into protoplasts by means of a two step procedure (Machtelinckx 1987). First, yeasts were suspended at a concentration of 200 mg wet weight/mL in a sterilized medium containing Na_2EDTA (0.05 M) and Tris-buffer (0.2 M; pH 8). After addition of 2-mercapto-ethanol (2% volume/volume) the yeast was incubated for 30 minutes at 30 C. Pretreated yeast was collected and washed with protoplasting medium comprising a phosphate-citrate buffer (KH_2PO_4 0.08 M; Na_3-citrate 0.016 M; pH 5.8) and KCl (0.6 M). A yeast pellet of 4 g wet weight was resuspended in 16 mL protoplasting medium to which 3 mL of an enzyme solution (Novozym 234, Novo; 40 mg/mL) was added. Conversion into protoplasts was completed after approximately 30 minutes of incubation at 30 C, whereupon the cells were separated from the enzyme mixture by centrifugation at 3,000 rpm. Yeast protoplasts could be stored in seawater at 4 C for at least one week without cell lysis.

Chemical treatment consisted of the first step in the previous procedure followed by washing the yeast cells three times with filtered seawater. This was an attempt to improve digestibility of the yeast without removing the cell wall, but by making the latter permeable for the digestive enzymes of *Artemia.* Finally, autoclaved yeast was prepared by autoclaving a suspension of fresh baker's yeast in a steam-boiler at 122 C and 1 atm for 45 minutes.

Artemia Culture Conditions

Great Salt Lake *Artemia* (Sandcrs Brinc Shrimp Co., lot 185-0) were hatched in 0.2-μm filtered artificial seawater (35 ppt salinity) at 25 C (Sorgeloos et al. 1986). Seawater was prepared according to the formula of Dietrich and Kalle (Kinne 1971). After 24 h (on day 1) freshly hatched nauplii were transferred to cylindroconical Falcon tubes (Falcon Blue Max 2070) containing 50 mL artificial seawater which was gently aerated by means of a Pasteur pipet. On the fourth day of culture, the initial density of 25 larvae/50 mL medium was reduced by transferring *ad random* 15 of the surviving nauplii to 50 mL fresh seawater. Cultures were kept under continuous darkness at 25 $\pm$ 1 C.

The live alga *Dunaliella tertiolecta* Butch, cultured according to Vanhaecke (1983) and known to be an excellent food for *Artemia,* was used as a reference diet. This algal diet was fed once a day according to the feeding schedule given in Table 1, which is based on the feeding rate of Vanhaecke and Sorgeloos (1980) in 10 mL test tubes. Dosage of the yeast diets were derived from the previous schedule by substituting three or more yeast cells for one algal cell (experiment 1 and 2, respectively 3). All cell counts were performed with a Bürker haemocytometer.

Experimental Design and Data Analysis

In the first experiment, protoplasts and chemically treated yeast cells were compared to untreated yeast in order to evaluate the effect on digestibility of enzymatic and chemical treatment. The second set of experiments evaluated the effectiveness of the chemical treatment with the commercially available baker's yeast. This study also examined the effect of autoclaving on the digestibility of the yeast. Laboratory-cultured *S. cerevisiae* was used to evaluate the effect of culture age on the susceptibility to diges-

tion by *Artemia* and on the efficiency of the chemical treatment. Finally, tests were run to determine the optimal feeding schedule for chemically treated Bruggeman's yeast under these culture conditions. Each treatment was run in six replicates.

Artemia survival and mean length were determined on day 4 and 8 for each test tube. Length from top of head to the base of the caudal furca was measured after fixation of the brine shrimp in lugol solution (Sorgeloos et al. 1986), using a dissecting microscope equipped with a drawing mirror. On day 4 an estimation of the length was obtained by measuring the remaining larvae in each treatment.

The data were analyzed statistically with a one-way analysis of variance (ANOVA). Tukey's honestly significant difference (HSD) method was used to detect significant differences between the means at the significance level of $P \leq 0.05$. Prior to analysis the data were checked for homoscedasticity and normality using Hartley's test and the Kolmogorov-Smirnov test, respectively. In some cases departure from the assumptions of analysis of variance were rectified by transformation of the original data (Sokal and Rohlf 1981).

Results

For all experiments, mean data and corresponding standard deviations for survival and length on day 4 and 8 are given in Table 2. The multiple range test for experiment 1 indicates that the untreated yeast diet yielded significantly lower growth and survival of the *Artemia* cultures compared to the other three diets. Among these latter treatments no differences could be detected except for higher survival of brine shrimp fed *Dunaliella,* and poor growth of *Artemia* fed yeast protoplasts compared to those fed chemically treated yeast. Microscopic examination of the fecal material produced by the larvae fed untreated baker's yeast revealed intact yeast cells. Staining with methylene blue showed that most of these cells still contained their cytoplasmatic contents after passage through the digestive system (Fig. 1). By contrast, fecal pellets of *Artemia* grown on treated diets were acellular and fine-granular (Fig. 2), revealing efficient digestion.

TABLE 1. *Feeding regime for 1-week culture of* Artemia *on* Dunaliella *in 50 ml Falcon tubes.*

Day	Number of *Artemia* per tube	Number of cells per larva ($\times 10^4$)	µl of stock suspension (18×10^6/ml) per vessel
1	25	15	210
2	25	30	420
3	25	30	420
4	15	30	250
5	15	45	380
6	15	45	380
7	15	60	500

ANOVA for data obtained in experiment 2 showed a significant effect of yeast culture age on digestibility. On day 8 length and survival of *Artemia* fed exponential-phase yeast were significantly higher in comparison to those fed the stationary-phase yeast. Chemical treatment significantly improved *Artemia* growth when applied to log-phase yeast and caked yeast, but was ineffective in promoting digestion of the stationary-phase yeast. A diet of autoclaved caked yeast resulted in significantly larger *Artemia* and higher survival rates.

The third set of experiments did not detect any significant difference between the results obtained by substituting one *Dunaliella* cell by 3, 4 or 5 yeast cells, respectively. Only the lowest food dosage, where one algal cell was replaced by one yeast cell, resulted in significant differences in growth and survival on day 8.

The *Dunaliella* diet proved to be a stable internal reference for this culture test; no significant ($P > 0.10$) deviations in *Artemia* growth and survival were noted among the three experiments.

Discussion

Extremely poor survival and growth of *Artemia* cultured on fresh baker's yeast

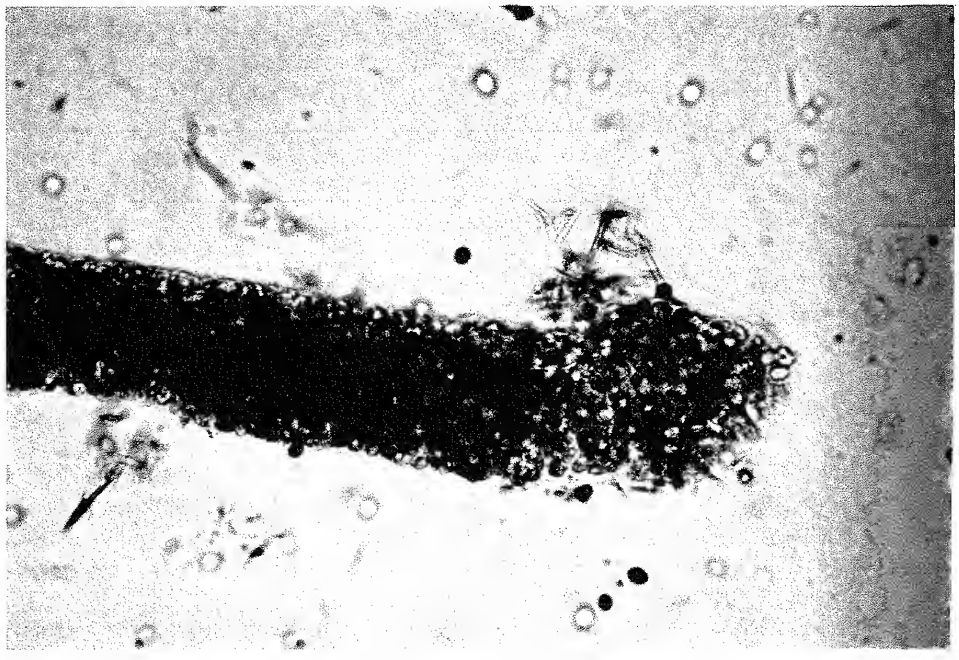

FIGURE 1. *Fecal pellet of* Artemia *fed fresh baker's yeast. Methylene blue colors cytoplasmatic contents (dark stained) of intact yeast cells. Non-stained material visible in this photograph reveals the presence of live yeast cells (×400).*

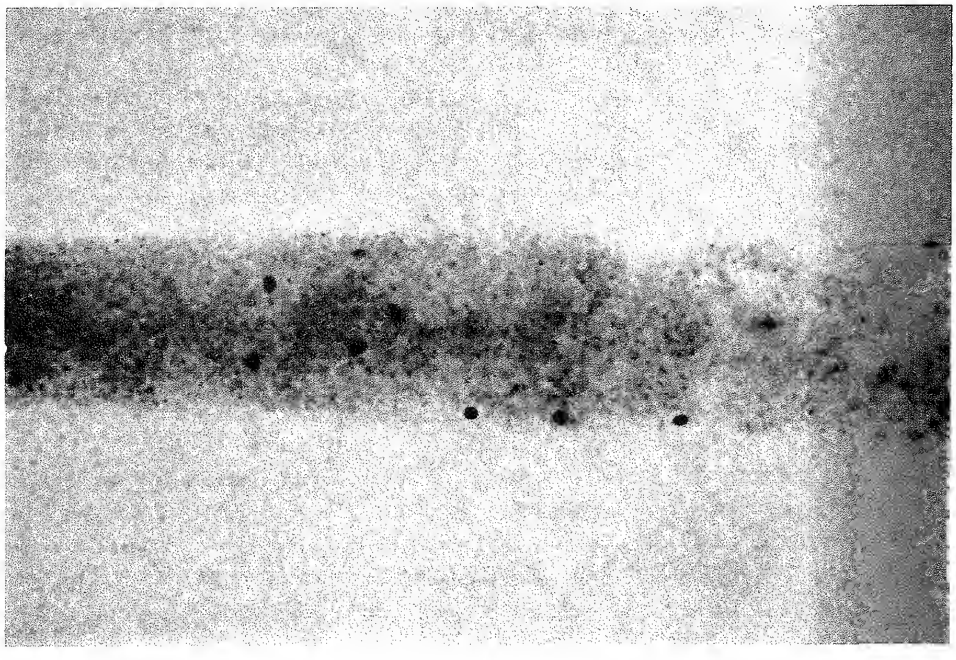

FIGURE 2. *Fecal pellet of* Artemia *fed chemically treated baker's yeast. Staining with methylene blue reveals only a few intact (dark stained) yeast cells. The bulk of the fecal material consists of yeast cell wall debris (×400).*

TABLE 2A. *Growth and survival of* Artemia *fed different yeast preparations. Data are presented as mean and standard deviation (SD) of six replicates. Unlike superscripts denote significant differences (ANOVA, Tukey HSD test* $P \leq 0.05$*).*

		Day 4				Day 8			
		Survival[1] (%)		Length (mm)		Survival[2] (%)		Length (mm)	
Code	Feeding regime	Mean	SD	Mean	SD	Mean	SD	Mean	SD
EXP 1									
1	Untreated baker's yeast	64.7^{b}	11.7	1.31^{b}	0.06	29.4^{c}	7.5	2.91^{c}	0.10
2	Yeast protoplasts	86.7^{a}	6.5	1.77^{a}	0.10	66.7^{b}	9.5	3.98^{a}	0.31
3	Chemically treated yeast	84.7^{a}	7.3	1.63^{a}	0.17	68.0^{b}	12.9	4.63^{b}	0.26
4	*Dunaliella tertiolecta* (internal reference)	94.4^{a}	6.7	1.80^{a}	0.15	94.6^{a}	5.5	$4.24^{a,b}$	0.18
EXP 2									
1	Caked yeast	76.7^{a}	9.6	1.10	—	$25.7^{a,b}$	19.8	1.56^{a}	0.08
2	Exponential-phsae yeast	$80.7^{a,b}$	8.5	1.43	—	$52.2^{b,c}$	18.0	2.93^{c}	0.54
3	Stationary-phase yeast	76.0^{a}	10.4	1.17	—	13.3^{a}	11.2	$2.11^{a,b}$	0.13
4	Caked yeast, treated	96.0^{c}	4.4	1.67	—	$53.3^{b,c}$	5.8	4.52^{d}	0.28
5	Exponential-phsae yeast, treated	$83.3^{a,b,c}$	4.7	1.88	—	61.0^{c}	7.9	5.00^{d}	0.25
6	Stationary-phase yeast, treated	$84.7^{a,b,c}$	6.4	1.42	—	10.0^{a}	3.3	$2.63^{b,c}$	0.74
7	Caked yeast, autoclaved	$91.3^{b,c}$	3.9	1.52	—	36.3^{c}	20.2	$2.51^{b,c}$	0.09
8	*Dunaliella tertiolecta* (internal reference)	94.7^{c}	5.5	1.72	—	94.3^{d}	7.8	4.22^{d}	0.08

[1] Survival day 1 = 100%.
[2] Survival day 4 = 100%.

clearly show that this diet is an inadequate food for brine shrimp. The observation of intact yeast cells in the fecal material of *Artemia* fed fresh baker's yeast reveal a problem with digestibility of this SCP. Gibor (1956) also observed viable *Stichococcus* sp. in fecal pellets of *Artemia* and attributed the low nutritional value of this alga to its low

TABLE 2B. *Growth and survival of* Artemia *fed chemically treated yeast according to different feeding regimes (dosages 1, 3, 4, and 5 correspond with the replacement of one algal cell by 1, 3, 4, or 5 yeast cells). Data are presented as mean and standard deviation (SD) of six replicates. Unlike superscripts denote significant differences (ANOVA, Tukey HSD test* $P \leq 0.05$*).*

		Day 4				Day 8			
		Survival[1] (%)		Length (mm)		Survival[2] (%)		Length (mm)	
Code	Feeding regime	Mean	SD	Mean	SD	Mean	SD	Mean	SD
1	dosage 1	98.0^{a}	4.9	1.36	—	18.8^{c}	8.7	2.89^{b}	0.25
2	dosage 3	94.7^{a}	5.5	1.84	—	48.7^{b}	13.0	4.56^{a}	0.37
3	dosage 4	98.0^{a}	2.2	1.85	—	57.5^{b}	17.1	4.91^{a}	0.42
4	day 1–3:dosage 3 day 4–7:dosage 4	96.7^{a}	3.0	1.82	—	65.5^{b}	15.4	4.76^{a}	0.25
5	day 1–3:dosage 3 day 4–7:dosage 5	96.7^{a}	6.4	1.73	—	63.2^{b}	12.4	4.58^{a}	0.22
6	*Dunaliella tertiolecta*; dosage 1 (internal reference)	98.4^{a}	2.2	2.04	—	93.4^{a}	9.4	4.37^{a}	0.30

[1] Survival day 1 = 100%.
[2] Survival day 4 = 100%.

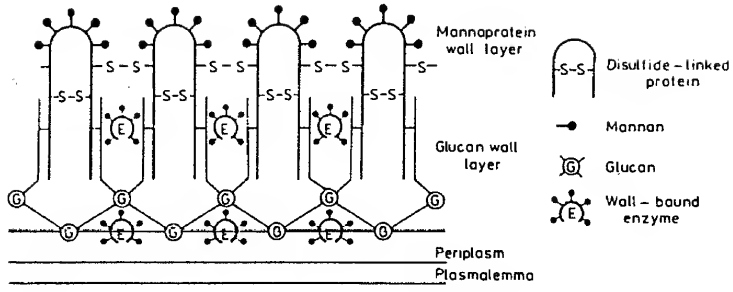

FIGURE 3. *Hypothetical model of the cell wall structure of Saccharomyces cerevisiae (Farkas 1985).*

digestibility. Because the nutritional value of the yeast improved after removal of the cell wall by enzymatic digestion, it is apparent that the primary limiting factor for using baker's yeast as a food for brine shrimp is the lack of digestion of the cell envelope.

Complete breakdown of the cell wall does not seem to be necessary for rendering the cytoplasmatic contents of the yeast cell accessible to the digestive system of *Artemia.* In fact, better growth performance was obtained after 8 days of culture when feeding chemically treated yeast than when feeding protoplasts. Thiol treatment is often applied as a preparatory step for the isolation of yeast protoplasts for use in biochemical and microbiological studies (Davis 1985). It is claimed that thiol compounds cleave the disulfide linkages in the cell wall, making it more permeable and susceptible to enzymatic degradation (Kidby and Davies 1970; Bacon 1973; Villaneuva et al. 1973). A hypothetical model of the cell wall structure of *Saccharomyces cerevisiae* (Fig. 3) indicates that two layers of wall material can be recognized. The outer layer consists of a mannoprotein complex in which the individual molecules are cross-linked by disulfide bridges made between their protein moieties. Farkas (1985) hypothesized that this cross-linking forms a barrier to penetration of extracellular glucanases into the internal glucan layer, which is the main structural constituent of the cell wall. For this reason, a mycolytic enzyme mixture should contain at least two components: first, an agent which increases the permeability of the mannoprotein layer and, second, an agent which dissolves the internal glucan microfibrils. The first component can be a mannanase (McLellan and Lampen 1968) or a specific proteolytic enzyme and may be substituted by treatment with 2-mercaptoethanol (Scott and Schekman 1980).

Bacon et al. (1965) demonstrated that autoclaving facilitates digestion of whole yeast cells by microbial enzymes. Significantly better growth of *Artemia* fed autoclaved yeast compared to untreated yeast confirms that autoclaving affects the yeast cell wall in such a way that the yeast is more susceptible to digestion. However, the lower *Artemia* growth obtained with the autoclaved yeast in comparison to the thiol treated yeast could be due to the extreme conditions of the heat treatment, which probably caused lysis of part of the yeast cells during the culture experiment. Consequently, the cells had lost some of their nutrients and water quality was affected.

In vitro experiments have shown that *Saccharomyces cerevisiae,* during its transition from exponential to stationary growth phase, builds up a resistance to enzymatic breakdown (Shahin 1972; Deutch and Parry 1974). Moreover, Schwencke et al. (1977) revealed that culture age of yeast influenced effectiveness of the thiol pretreatment on protoplast isolation. This present study is the first confirmation of this effect for *in vivo* digestion.

As mandibular grinding of captured food particles is unknown for *Artemia,* it may be supposed that requirements for the diges-

tion of yeast *in vitro* are similar to those for the enzymatic breakdown in the digestive tube. Little is known about the spectrum of enzyme activities in the digestive tract of the brine shrimp. Telford (1970) found β-glucanase activity in a whole-body homogenate of *Artemia* but could not detect any mannanase activity. The authors of the present study have confirmed his findings in a qualitative assay on these two enzymes (unpublished data). The inability of *Artemia* to grow on baker's yeast strongly suggests that the digestive enzymes of *Artemia* cannot penetrate the outer mannoprotein layer of the yeast cell wall. Absence of mannanase activity and the efficient digestion of yeast after thiol treatment or autoclaving confirm this hypothesis.

Reports on the more or less successful culture of brine shrimp on yeasts (Bond 1937; Weisz 1946; Bowen 1962; Shimaya et al. 1967; Kawano et al. 1976; Bowen et al. 1985; Blanco Rubio 1987; James et al. 1987) are not necessarily in contradiction with these current findings. In fact, during preliminary experiments in which up to 75% of *Dunaliella* cells were substituted with fresh baker's yeast, culture results were obtained which were sometimes comparable to those of the 100% algal diet. Many of the results reported in the literature could be explained by a contamination of the culture with algae or other micro-organisms. Moreover, supplying yeast to the medium induces development of a microflora which may play an essential role in the nutritional properties of the diet for *Artemia* (Douillet 1987). This effect may be the result of supplementation of nutrients and/or the improvement of digestibility characteristics. In addition, it has been demonstrated that the structural organization, composition and digestibility of the yeast cell wall differ considerably with species (Bartnicki-Garcia 1968; Kreger-Van Rij and Veenhuis 1971) and strain (Rost and Venner 1965; Kaneko et al. 1973). Finally, susceptibility to enzymatic degradation may also be influenced by age of the culture and by medium composition (Killick 1971; Kratky et al. 1975) or various other culture conditions (Kaneko et al. 1973).

In conclusion, the ineffectiveness of baker's yeast as a diet for *Artemia* appears to be mainly due to poor digestibility rather than its nutritional composition. Moreover, this problem can be solved without affecting the structural backbone of the yeast cell wall. In this way, the suitable characteristics of yeast (i.e., individual cells that do not lose nutrients into the culture medium) can be retained and yeast might eventually be used as a diet for intensively cultured brine shrimp and aquatic filter-feeders, e.g., bivalves and penaeid shrimp larvae. Our test system with *Artemia* can be used as a model system for evaluating different SCP preparations as a potential food source for these commercially important aquaculture species.

Acknowledgments

Special thanks are due to Prof. Dr. ir. E. Vandamme and Dr. ir. L. Machtelinckx (Laboratory of General and Industrial Microbiology—Faculty of Agricultural Sciences—State University of Ghent) for their advice and assistance concerning the preparation of yeast protoplasts.

This study has been supported by the Belgian Ministry of Science Policy (00A contract 87/93-118) and the Belgian National Fund for Scientific Research NFWO (P.S. and P.C. are a senior research associate and a research assistant, respectively, with the NFWO).

Literature Cited

Bacon, J. S. D. 1973. Contribution of β-glucanases to lysis of cell walls. Pages 61–73 *in* J. R. Villaneuva, I. Garcia Acha, S. Gascon and F. Uruburu, editors. Yeast, mould and plant protoplasts. Academic Press, New York, USA.

Bacon, J. S. D., B. D. Milne, I. F. Taylor and D. M. Webley. 1965. Features of the cell-wall structure of yeast revealed by the action of enzymes from a non-fruiting myxobacterium (*Cytophaga johnsonii*). Biochemical Journal 95:28c–30c.

Bartnicki-Garcia, S. 1968. Cell wall chemistry, mor-

phogenesis, and taxonomy of fungi. Annual Review of Microbiology 22:87–108.

Blanco Rubio, J. C. 1987. Intensive rearing *Artemia salina* larvae on inert food: yeast of Torula (*Candida utilis*). Cuad. Marisq. Publ. Tec. 12:565–568.

Bond, R. M. 1937. A method for rearing *Artemia salina*. Pages 205–206 *in* P. S. Galtoff, F. E. Lutz, P. S. Welch and G. G. Needham, editors. Culture methods for invertebrate animals. Dover Publications, New York, USA.

Bowen, S. T. 1962. The genetics of *Artemia salina*. I. The reproductive cycle. Biological Bulletin 122(1):25–32.

Bowen, S. T., E. A. Fogarino, K. N. Hitchner, G. L. Dana, H. S. Chow, M. R. Buoncristiani and J.R. Carl. 1985. Ecological isolation in *Artemia*: population differences in tolerance of anion concentrations. Journal of Crustacean Biology 5(1):106–129.

Davis, B. 1985. Factors influencing protoplast formation. Pages 45–71 *in* J. F. Peberdy and L. Ferenczy, editors. Fungal protoplasts. Applications in biochemistry and genetics. Marcel Dekker, Inc., New York, USA.

Deutch, C. E., and J. M. Parry. 1974. Sphaeroplast formation in yeast during the transition from exponential phase to stationary phase. Journal of General Microbiology 80:259–268.

Douillet, P. 1987. Effect of bacteria on the nutrition of the brine shrimp *Artemia* fed on dried diets. Pages 295–308 *in* P. Sorgeloos, D. A. Bengtson, W. Decleir and E. Jaspers, editors. *Artemia* research and its application. Volume 3. Ecology, culturing, use in aquaculture. Universa Press, Wetteren, Belgium.

Epifanio, C. E. 1979. Comparison of yeast and algal diets for bivalve molluscs. Aquaculture 16:187–192.

Farkas, V. 1985. The fungal cell wall. Pages 3–29 *in* J. F. Peberdy and L. Ferenczy, editors. Fungal protoplasts. Applications in biochemistry and genetics. Marcel Dekker, Inc., New York, USA.

Fukusho, K. 1980. Mass-production of a copepod, *Tigriopus japonicus* in combination culture with a rotifer *Brachionus plicatilis*, fed Omega yeast as a food source. Bulletin of the Japanese Society of Scientific Fisheries 46:625–629.

Gibor, A. 1956. Some ecological relationships between phyto-and zooplankton. Biological Bulletin 111(2):230–234.

Hedenskog, G. and H. Mogren. 1973. Some methods for processing single cell protein. Biotechnology and Bioengineering 15:129–142.

Hirayama, K. 1987. A consideration of why mass culture of the rotifer *Brachionus plicatilis* with baker's yeast is unstable. Hydrobiologia 147:269–270.

Hirayama, K. and T. Watanabe. 1973. Fundamental studies on physiology of rotifer for its mass culture 4. Nutritional effect of yeast on population growth of rotifer. Bulletin of the Japanese Society of Scientific Fisheries 39:1129–1133.

James, C. M., T. S. Abu-Rezeq and P. Dias. 1987. Production of *Artemia* biomass for feeding marine fish larvae (Abstract). Page 349 *in* P. Sorgeloos, D. A. Bengtson, W. Decleir and E. Jaspers, editors. *Artemia* research and its application. Volume 3. Ecology, culturing, use in aquaculture. Universa Press, Wetteren, Belgium.

Johnson, D. A. 1980. Evaluation of various diets for optimal growth and survival of selected life stages of *Artemia*. Pages 185–192 *in* G. Persoone, P. Sorgeloos, O. Roels and E. Jaspers, editors. The brine shrimp *Artemia*. Volume 3. Universa Press, Wetteren, Belgium.

Johnson, E. A., T. G. Villa, and M. J. Lewis. 1980. *Phaffia rhodozyma* as an astaxanthin source in salmonid diets. Aquaculture 20:123–134.

Kaneko, T., K. Kitamura and Y. Yamamoto. 1973. Susceptibilities of yeasts to cell wall lytic enzyme of *Arthrobacter luteus*. Agricultural and Biological Chemistry 37(10):2295–2302.

Kawano, T., H. Kojima, H. Ohosawa and K. Morinaga. 1976. Feed and method of aquianimals cultivation. Unites States Patent Number 3,939,279. February 17, 1976.

Kidby, D. K. and R. Davies. 1970. Invertase and disulfide bridges in the yeast cell wall. Journal of General Microbiology 61:327–333.

Kihlberg, R. 1972. The microbe as a source of food. Annual Review of Microbiology 26:427–466.

Killick, K. A. 1971. Modification of the composition and structure of the yeast cell wall by culture in the presence of sulfur amino acids. Journal of Bacteriology 106(3):931–937.

Kinne, O. 1971. Marine ecology. Volume 1. Environmental factors. Part 2. John Wiley and Sons, New York, USA.

Kratky, Z., P. Biely and S. Bauker. 1975. Mechanism of 2-deoxy-D-glucose inhibition of cell wall polysaccharide and glycoprotein biosynthesis in *Saccharomyces cerevisiae*. Biochemistry 54:459–467.

Kreger-Van Rij, N. J. W. and M. Veenhuis. 1971. A comparative study of the cell wall structure of basidiomycetous and related yeasts. Journal of General Microbiology 68:87–95.

Lemilinaire, C. 1984. Contribution à l'étude du besoin en acides gras essentiels pour la larve du turbot (*Scophthalmus maximus*) pendant la phase d'alimentation avec le Rotifère *Brachionus plicatilis* O.F. Müller. Thèse 3ème cycle, Bretagne Occidentale.

Machtelinckx L. 1987. Protoplastfusie bij gisten en moleculair-genetische karakterisatie van de fusiehybriden. Doctoral thesis. State University of Ghent, Ghent, Belgium.

McLellan, W. L. and J. D. Lampen. 1968. Phosphomannanase (PR-factor), an enzyme required

for the formation of yeast protoplasts. Journal of Bacteriology 95:967–974.

Mitsuda, H., K. Yasumoto and H. Nakamura. 1969. A new method for obtaining protein isolates from chlorella algae, torula yeasts, and other microbial cells. Engineering and unconvential protein production 65:93–103.

Nimmannit, S. and S. Assawamunkong. 1985. Study on yeast as feed for marine organisms (brine shrimps). Proceedings of Living Aquatic Resources, 7–8 March, Chulalongkorn University.

Omstedt, P., V. von der Decken, G. Hedenskog and H. Mogren. 1975. Effect of processing on the nutritive value of *Saccharomyces cerevisiae, Scenedesmus obliquus,* and *Spirulina platensis* measured by protein synthesis *in vitro* in rat skeletal muscle. Pages 553–558 *in* S. R. Tannenbaum and D. I. C. Wang, editors. Single-cell protein II. The MIT Press, Cambridge, Massachusetts, USA.

Rost, K. and H. Venner. 1965. Enzymatische Zellwand Verdauung bei Hefen durch Schnechenenzym. Archiv für Mikrobiologie 51:122–129.

Schwencke, J., N. Magana-Schwencke and J. Laporte. 1977. Yeast protoplasts from stationary and starved cells: preparation, ultrastructure and vacuolar development. Annales de Microbiologie 128(A–1):3–18.

Scott, J. H. and R. Schekman. 1980. Lyticase: endoglucanase and protease activities that act together in yeast cell lysis. Journal of Bacteriology 142(2):414–423.

Shahin, M. M. 1972. Relationship between yield of protoplasts and growth phase in *Saccharomyces.* Journal of Bacteriology 110:769–771.

Shimaya, M., A. Kanazawa and K. Kashiwada. 1967. Studies in the utilization of marine yeast-I. Culture of *Artemia* and *Daphnia* by marine yeast. (Japanese). Memoirs of the Faculty of Fisheries Kagoshima University 16:34–39.

Sokal, R. R. and F. J. Rohlf. 1981. Biometry. W. H. Freeman and Company, San Francisco, California, USA.

Sorgeloos, P., P. Lavens, P. Léger, W. Tackaert and D. Versichele. 1986. Manual for the culture and use of brine shrimp *Artemia* in aquaculture. *Artemia* Reference Center, Faculty of Agriculture, State University of Ghent, Belgium.

Talloen, M. 1978. The use of locally available food for the mass-culture of the brine shrimp *Artemia salina.* Bulletin of the Jepara Brackishwater Aquaculture Development Center.

Telford, M. 1970. Comparative carbohydrase activities of some crustacean tissue and whole animal homogenates. Comparative Biochemistry and Physiology 34:81–90.

Urban, E. R. and C. J. Langdon. 1984. Reduction in costs of diets for the American oyster, *Crassostrea virginica* (gmelin), by the use of non-algal supplements. Aquaculture 38:277–291.

Vanhaecke, P. 1983. Vergelijkende studie van diverse geografische rassen van het pekelkreeftje, *Artemia,* ter verbetering van zijn gebruik in de aquakultuur. Doctoral thesis. State University of Ghent, Ghent, Belgium.

Vanhaecke, P. and P. Sorgeloos. 1980. International study on *Artemia.* IV. The biometrics of *Artemia* strains from different geographical origin. Pages 393–405 *in* G. Persoone, P. Sorgeloos, O. Roels, and E. Jaspers, editors. The brine shrimp *Artemia.* Volume 3. Ecology, culturing, use in aquaculture. Universa Press, Wetteren, Belgium.

Villaneuva, J. R., M. Gacto and J. M. Sierra. 1973. Enzymatic composition of a lytic system from *Micromonospora chalcea* AS. Pages 3–24 *in* J. R. Villaneuva, I. Garcia Acha, S. Gascon and F. Uruburu, editors. Yeast, mould and plant protoplasts. Academic Press, New York, USA.

Weisz, P. B. 1946. The space-time pattern of segment formation in *Artemia salina.* Biological Bulletin 91(2):119–140.